Resistance to Penicillin and Identification of Penicillinase Producing *Neisseria gonorrhoeae* from Clinical Isolates in Thailand

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OBJECTIVES

1. To monitor selected clinic populations reporting symptoms of gonococcal disease and having infections confirmed by laboratory culture.

2. To identify those isolates with resistance to penicillin (RSP).

3. To perform determinations of penicillin minimum inhibitory concentrations (MIC) from RSP isolates.

4. To identify the penicillinase-producing *Neisseria gonorrhoeae* (PPNG) and correlate with MIC findings.

Penicillin has historically proven an effective and low cost therapy for the treatment of uncomplicated gonococcal infections. Many new antibiotics are also active against gonococcal infections. Beta-lactamase positive strains of *Neisseria gonorrhoeae* have in general been shown to demonstrate in-vitro resistance to penicillin G, ampicillin, amoxicillin and carbencillin. Strains of gonococci isolated from East Asia have shown varied susceptibility to methicillin, oxacillin, nafcillin, cloxacillin and dicloxacillin whether the strain was identified as a penicillinase-producing or not. However, the overall trend is towards constant or increasing antibiotic resistance (13). The surveillance of penicillin susceptibility and beta-lactamase activity of isolates does provide one guide for therapeutic recommendations and contributes information to the apparent trend of resistance.

From 1972, an increasing resistance to penicillin by *N. gonorrhoeae* has been demonstrated in Bangkok (10). Penicillinase-producing *Neisseria gonorrhoeae* (PPNG) identifications has been reported from 27 countries in Europe, Asia, Africa, Oceania, and North America to the World Health Organization (1). Epidemiological evidence suggests two separate focal origins of PPNG strains, the Far East and parts of West Africa (7). The potential of plasmid-initiated resistance

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as a factor with antibiotics other than penicillin, the risk of such plasmids entering organisms other than the gonococci, and the spread of resistant strains to geographic areas of low incidence has made penicillin sensitivity and PPNG identification a world-wide public health objective (6).

METHODS : A total of 230 male and 175 female cases were studied, beginning in April 1978. All patients attended the Phra Mongkutklao Royal Thai Army Hospital venereal disease clinic or the Ban Chiwi clinic of the Bangrak (Public Health) Hospital for venereal disease examination. Patients were selected as demonstrating clinical symptoms of *Neisseria gonorrhoeae* infection or recent history of poor response to therapy (12). Male patient specimens were taken as urethral exudate and female specimens as cotton bud swabs from the cervical area. Specimens were immediately prepared for a microscopic gram stain examination and streaked on to Thayer-Martin agar (11) plates which were incubated for up to 72 hours at 37°C and 10% carbon dioxide. Gonococci were identified by gram stain, colonial morphology, oxidase reaction, and sugar fermentation (4).

Culture confirmed isolates were studied for the production of penicillinase using a penicillin disc diffusion technique, isolates being streaked on a cultured lawn of penicillin susceptible *Staph. aureus* strain (13), and by the chromagenic cephalosporin test (5). Positive agreement between the techniques was required for PPNG identification.

Minimum inhibitory concentrations to penicillin were determined by plate dilution (10) using standardized culture suspensions and Thayer-Martin plate serial dilutions of penicillin G in concentrations of 0.06 through 24 micrograms per milliliter. Isolates with an MIC greater than 24 µgm/ml were reported as such and those with less than 0.6 were considered susceptible.

RESULTS : The pattern of penicillin G MIC activity for the 405 isolates (Table 1; Figure 1) shows the trend of a simple gaussian-like distribution. Quarter year data (Table 2; Figure 2) did not show any notable variation over the year. The mean MIC and one standard error (SE), excluding penicillinase-producing isolates, for males was 0.799 $\pm$ 0.039; females 0.812 $\pm$ 0.032 and overall 0.805 $\pm$ 0.022. Seventyfive isolates 52 from males and 23 from females, were identified as PPNG. All isolates with an MIC greater than or equal to 6 µgm/ml were identified as penicillinase-producing (Table 1). The percentage of PPNG isolates was 22.6 percent of male isolates, 13 percent of female and 18.5 percent of the total 405.

Treatment regimens used in Bangkok on suspected venereal disease patients include spectinomycin (2-4 gm), ampicillin and probenecid (3.5 gm), procaine-penicillin G and probenecid (4.8-6 million U, IM), or kanamycin (2 gm). Selection of the treatment regimen was based on clinical judgement, response and drug availability. The ampicillin or the procaine penicillin G and probenecid regimens were the most commonly prescribed. Studies in the United States have shown that PPNG strains have higher MICs for penicillin, ampicillin, erythromycin, tetracycline, and spectinomycin (9). The mean penicillin MIC in µcg/ml, reported in Thailand during 1972, 1973, and 1974 (10) was 0.348, 0.432 and 0.63, respectively. Our two previous studies of selected clinical populations, excluding PPNG isolates (3, 2) exhibited a mean MIC and SE of 0.688 $\pm$ 0.044 and 0.883 $\pm$ 0.037. In our present study, the mean penicillin MIC and SE was 0.805 $\pm$ 0.022. During

1972-74, data were calculated deleting all MIC values above 1.2 µgm/ml. If this exclusion were applied to our 1979 data, it would represent over 25 percent of the values used for calculation purposes. We reported during 1977 that 8 percent of our 105 study isolates were beta-lactamase positive and during 1978 that 9 percent of 182 isolates were beta-lactamase positive. Of the present 405 cases, 75 (18.5%) were PPNG positive and had a MIC indicating penicillin resistance.

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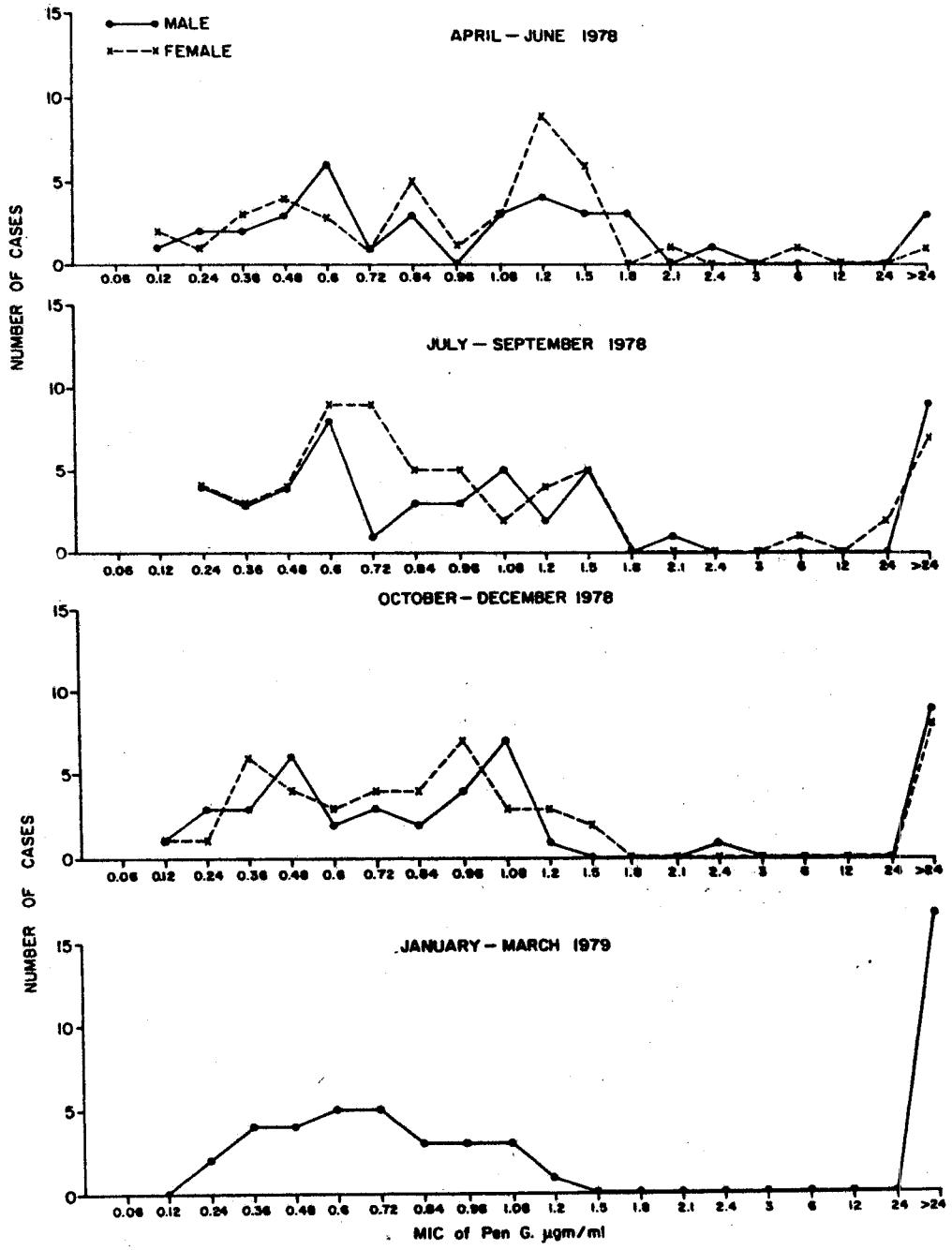
Table 1. Pattern of Penicillin G MIC (µgm/ml) Activity

MIC (Pen. G µgm/ml)	Male (n = 230) Cases	Female (n = 175) Cases	β-lactamase
0.06	0	0	-
0.12	3	3	-
0.24	13	7	-
0.36	18	15	-
0.48	23	19	-
0.60	26	19	-
0.72	17	17	-
0.84	17	14	-
0.96	12	16	-
1.08	21	10	-
1.2	9	15	-
1.5	9	13	-
1.8	4	2	-
2.1	3	2	-
2.4	2	0	-
3.0	1	0	-
6.0	1	3	+
12.0	0	0	
24.0	1	2	+
>24.0	50	18	+
TOTAL	230	175	

Table 2. MIC by Months

MIC	Mar 78		Apr-Jun 78		Jul-Sep 78		Oct-Dec 78		Jan-Mar 79		Apr-Jun 79		July 79	
µgm/ml	M	F	M	F	M	F	M	F	M	F	M	F	M	F
0.12			1	2			1	1			1			
0.24	1		2	1	4	4	3	1	2	1	1			
0.36			2	3	3	3	3	6	4		4		1	
0.48		2	3	4	4	4	6	4	4	5	6			
0.6		5	6	3	8	9	2	3	5	2	5		1	
0.72	4	2	1	1	1	9	3	4	5		3			
0.84	2		3	5	3	5	2	4	3		3		1	
0.96	1	3		1	3	5	4	7	3		1			
1.08	1	2	3	3	5	2	7	3	3		1			
1.2			4	9	2	4	1	3	1		1		1	
1.5			3	6	5	5		2						
1.8	2	2	3											
2.1	2	1		1	1									
2.4			1				1							
3.0											1			
6.0		1	1	1		1								
12.0														
24.0						2					1			
>24	2	1	3	1	6	7	9	8	18	1	11		1	
TOTAL	15	19	36	41	45	60	42	46	48	9	39		5	

MINIMAL INHIBITORY CONCENTRATION (MIC) PATTERN (THREE MONTHS PERIOD)



MINIMAL INHIBITORY CONCENTRATION (MIC) PATTERN